IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Reissue Application of: U.S. Patent No. 5,750,338 Mark L. Collins *et al.* Reissue Serial No. 09/533,906 Reissue Application Filed: March 8, 2000 For: TARGET AND BACKGROUND CAPTURE METHODS WITH AMPLIFICATION FOR AFFINITY ASSAYS	Group Art Unit: Unassigned Examiner: Unassigned

DECLARATION OF MICHAEL M. HARPOLD, Ph.D.

ATTENTION: REISSUE LITIGATION BOX 7
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Michael M. Harpold, residing at 5920 N. Placita Tecolote, Tucson, Arizona 85718, do hereby declare as follows:

1. I have been Principal of EnkephaSys, involved in biomedical consulting, since 1999. From 1998-1999, I was Chief Scientific Officer and a Director of the National Center for Genome Resources, a non-profit research institute involved in computational biology and bioinformatics research. I was employed by SIBIA Neurosciences, Inc. (formerly The Salk Institute Biotechnology/Industrial Associates, Inc.) from 1981, serving as a founding Senior Staff Scientist from 1981-1982, as Director of Scientific Planning from 1982-1986, as Vice President for Scientific Planning from 1986-1990, and as Vice President, Research from 1990-1998. From

1973-present, I have conducted and directed research in a variety of areas of biological science, including molecular biology, nucleic acid hybridization, and DNA probe technologies. A copy of my *curriculum vitae* is attached hereto as Exhibit A.

2. I have read copies of the following documents relating to U. S. Patent No. 5,750,338 (the '338 patent'), which is the subject of the above-captioned reissue application:

U.S. Patent No. 5,750,338;

Prosecution file history of U.S. Serial No. 08/238,080, including the Declaration of David H. Persing, M.D., Ph.D., filed July 14, 1997; and

Preliminary Amendment filed with reissue application no. 09/533,906, dated March 8, 2000.

3. The '338 patent describes methods of detecting nucleic acid sequences that use capture of polynucleotide sequences on a solid phase support and non-specific amplification of the captured polynucleotide. I have been informed that the filing date of the first patent application that discloses this combination of steps and from which the '338 patent claims priority is December 21, 1987 (the "filing date").

4. I believe that the invention claimed in the '338 patent and its reissue application was known or obvious to one skilled in the art at the filing date of the application. I also believe that the specification of the '338 patent fails to convey to one of ordinary skill in the art that the inventors were in possession of the invention claimed in new claims 41-59. More specifically, the specification conveys that the applicants were only in possession of a method that employed *in vitro* non-specific amplification, whereas new claims 41-59 are not limited to non-specific amplification. In support of these opinions, in the following paragraphs, I will present a short

summary of the general state of the art at the filing date of the '338 patent application; my view of the level of skill in the art at the filing date of the '338 patent application; a discussion of disclosures in published references that were available to those skilled in the art at the filing date of the '338 patent application; and a discussion of what the specification would convey to one of skill in the art regarding the scope of the methods that the reissue applicants were in possession of at the filing date. I will also present my views of the state of the art in contrast to those expressed by Dr. David Persing in his declaration filed in 1997.

5. General State of the art at the filing date of the '338 patent application.

Following the discovery of reverse transcriptase (1970), and the development of cloning (1973) and DNA sequencing (1975), biological scientists began to study individual genes and DNA sequences associated with gene expression. It also became possible to develop DNA probe based methods for the detection of normal and mutant genes and for the detection and identification of infectious organisms using the techniques of molecular biology. The low frequency of individual genes in the genome (e.g., 1 in 40,000 human genes) and/or the low concentration of individual gene expression products in soluble cellular components presented technical problems for such studies and methods. Therefore, scientists were motivated to develop methods to separate and/or concentrate individual genes from other cellular components. In addition, scientists were motivated to amplify individual sequences to provide sufficient quantities for analysis and detection. Recombinant DNA cloning was one method that allowed scientists to isolate discrete DNA fragments from any organism and produce (amplify) large amounts of the same DNA for further characterization. Cloning itself was, however, a very laborious process when genomic DNA, or DNA transcribed from unfractionated messenger RNA, were used as the starting material. Often, tens of thousands or hundreds of thousands of

clones had to be screened to find those containing the desired sequence. See, *e.g.*, Maniatis et al., Molecular Cloning A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 1982), in Chapter 10, pages 309-362.

To increase the frequency of clones with a particular target sequence, scientists often isolated mRNA from tissue that specifically produced the target gene product. For example, mRNA was isolated from pancreatic islet cells to enrich for sequences encoding insulin. Methods were developed to separate mRNA from other RNA and proteins found in cellular extracts, such as by using hybridization of the poly(A) tail of mRNA to a complementary oligo(dT) sequence in chromatography, *i.e.*, capture on a solid support (see Maniatis et al., *supra*, Chapter 6). The isolated mRNA could then be converted to cDNA using reverse transcriptase, cloned and amplified by replication in bacterial cells. Thus, it was commonplace before the filing date for scientists to employ the combination of enrichment by target capture and amplification in order to generate detectable amounts of nucleic acid sequences of interest.

The application of molecular biological techniques to the diagnosis of disease has been recognized for decades. Many commonly used immunoassays for infectious agents employ a combination of target capture and amplification to enhance detection sensitivity. In so-called sandwich assays, a solid support containing a bound capture antibody is used to capture an antigen from the specimen being tested. The other sample components are washed away. Other antibodies, coupled with enzymes, bind to the captured antigen. After excess detection antibodies are washed away, an amplified signal is generated from the bound enzyme by its repeated production of a readily detected product. Immunoassays are members of the general class of ligand binding assays, in which a component characteristic of the infectious agent is bound specifically to a support (target capture) and detected. In cases where direct detection is

insufficiently sensitive, various amplification methods are commonly employed to amplify the signal generated from the presence of the captured ligand. Those skilled in the art recognized that hybridization assays were another form of ligand binding assay and that unique nucleic acid sequences present in infectious agents could be used to detect and identify them in the same way in which antigen:antibody reactions were employed. In these assays, which were commonly performed in a sandwich format, a labeled nucleic acid probe was used to detect the presence of a nucleic acid sequence indicative of the presence of the infectious organism.

In some infectious diseases, the number of organisms present may be small, and test sensitivity must be high to detect the majority of cases. As with immunoassays, scientists found that diagnostic tests based on hybridization between complementary DNA sequences often lacked sensitivity when the number of infectious agents in the sample was small because the signal over background that could be generated from a low number of hybrids was insufficient for reliable detection. Thus, hybridization assays were insensitive compared to tests in which the number of organisms was amplified by growing them in culture. To overcome the lack of sensitivity of DNA-based probe tests, scientists used amplification to enhance the signal obtainable from the probe:target hybrids. As with the antibodies used in immunoassays, probes could be labeled with enzymes. The enzyme label would repeatedly transform a substrate into product, thereby amplifying the signal from each hybrid formed. In this way, the sensitivity of detection was enhanced.

Thus, it was common practice in the art prior to December, 1987, to combine technologies, such as target capture, amplification and hybridization methods, to obtain specific and sensitive detection of sequences of interest.

6. I consider the level of ordinary skill in the art of molecular biology at the filing date of the '338 patent application to have been that of an individual with a Ph.D. in the biological sciences and two years of postdoctoral experience. Such experience would have allowed the individual to develop the skills of a molecular biologist or genetic engineer using the techniques of DNA and RNA isolation and characterization, cDNA synthesis, cloning, liquid and solid phase hybridization (including knowledge of the conditions influencing hybrid formation and stability), affinity chromatography, isotopic and non-isotopic labeling methods, DNA sequencing methods, and nucleic acid amplification, including, but not limited to, PCR.

7. Relevant techniques that were well known in the art at the filing date of the '338 application, based on published scientific articles and U.S. Patents, are summarized below. They have been grouped into sections related to Capture Technology, Amplification Technology, and the combination of Capture and Amplification Technology. These provide a more detailed basis for my opinion that one skilled in the art at the filing date of the '338 application would have been motivated to combine known techniques to produce the invention claimed in the '338 patent.

8. Capture Technology

Before the filing date, it was well known among those skilled in the art that a polynucleotide of interest could be purified from a sample by contacting it with a solid support that would bind the polynucleotide and then separating the bound target from the sample. Such a purification step would remove components in the sample that would otherwise prevent or inhibit the further manipulation (e.g., cloning or in vitro amplification) of the target polynucleotide.

It was standard practice for many years before 1987 to isolate poly(A)-tailed mRNA from a biological sample by contacting the sample with oligo(d)T bound to a solid support (e.g., cellulose) under conditions that permitted hybridization of the poly(A)$^+$ mRNA with the oligo(dT) moiety (see Maniatis et al., *supra*, Chapter 6). After separating the bound support and mRNA from the sample, the purified mRNA could then be released from the solid support and used in a variety of ways (e.g., in cDNA synthesis and/or molecular cloning). For example, Gaubatz et al., *Biochim. Biophys. Acta* 825:175-187 (1985) described the isolation of poly(A)$^+$ mRNA using oligo(dT)-cellulose chromatography followed by conversion of the purified mRNA into cDNA and further amplification using the Klenow fragment of DNA polymerase I. It was also well known in the art before December 1987 that sequence-specific capture of a target polynucleotide could be carried out by contacting a sample containing the target polynucleotide with a solid support and a capture probe that was capable of binding the solid support and that contained a sequence of nucleotides complementary to a specific sequence in the target polynucleotide. For example, *U.S. Patent No. 4,672,040* (Josephson) and *U.S. Patent No. 4,554,088* (Whitehead et al.) describe use of dispersible solid supports--magnetic beads--with bound polynucleotide capture probes for isolating target polynucleotides. By separating the magnetic beads with their adherent target polynucleotide using a magnet, the target polynucleotide is separated from other sample components capable of inhibiting or negatively affecting further manipulation, including detection, of the target polynucleotide.

Nucleic acid hybridization assays employing sequence-specific capture of target polynucleotides were also well known in the art before December 21, 1987. In these assays, target polynucleotide was first isolated from a sample by contacting the sample with a solid support having bound to it a capture probe with a sequence complementary to at least a portion

of the target under conditions that permit the target and probe to hybridize. After separating the bound target from the sample, the target could be detected (e.g., by using a labeled probe). Such assays are described in Polsky-Cynkin et al., *Clin. Chem.* 31(9):1438-1443 (1985) and *U.S. Patent No. 4,563,419* (Ranki et al.). Syvänen et al., *Nucleic Acids Res.*, 14: 5037-5048 (1986), describe a sandwich hybridization assay in which a biotin-containing capture DNA and ^{125}I-labeled probe were first hybridized to the target polynucleotide in solution and the target-probe-capture DNA complex was subsequently contacted with a solid support (i.e., streptavidin-agarose) and bound to the support via a streptavidin-biotin linkage through the biotinylated capture probe. The presence of the ^{125}I-labeled probe allowed detection of the bound complex. Because hybridization of probe to the target occurred in solution prior to capture on the solid support, greater capture efficiency was achieved using solution-phase hybridization kinetics.

9. Amplification Technology

Various methods for amplifying nucleic acids were well known in the art prior to the filing date. One skilled in the art would understand "amplify" as defined in the '338 patent (col. 2, lines 9-19) to encompass a wide variety of well known methods to either directly produce more copies of the captured polynucleotide or indirectly amplify the presence of the target polynucleotide. Direct amplification methods would include known methods of *in vitro* nucleic acid amplification, including cDNA synthesis, and cloning (i.e., additional target molecules are produced by inserting the target nucleic acid into host cells and growing the transformed cells). An example of indirect amplification would be cell-free gene expression (i.e., protein molecules are created by virtue of the presence of the target polynucleotide). In addition to these molecular biology techniques, other methods of target amplification were known before December 1987. In particular, all of the methods of amplification disclosed in the '338 patent were well known

then. Feinberg et al., *Anal. Biochem.* 132:6-13 (1983) had described use of random hexamer primers to initiate non-specific enzymatic amplification of polynucleotides (Examples 5 and 6 of the '338 patent). Blumenthal et al., *Proc. Natl. Acad. Sci. U.S.A.* 77:2601 (1980) had described use of the enzyme Q-beta (Qβ) replicase to amplify polynucleotides (Example 7 of the '338 patent). R. Burgess in RNA Polymerase (Cold Spring Harbor Press, (1976), pp. 69-100 had described use of RNA polymerase core enzyme to non-specifically replicate polynucleotides (Example 4 of the '338 patent). Besides the amplification methods described in the '338 patent, other methods of amplifying polynucleotides were well known in the art before the filing date. Gaubatz et al., *Biochim. Biophys. Acta* 825:175-187 (1985) had described a method of amplifying cDNA sequences by a polymerase-mediated strand displacement synthesis. PCR had been described in a number of publications before the filing date and in U.S. patents filed before December, 1987, including U.S. Patent No. 4,683,202 (Mullis).

10. Combination of Capture and Amplification Technology

In my opinion, the scientific literature had also taught prior to the filing date that it would be useful to combine the techniques of target capture and amplification to enrich and detect target molecules of interest in samples, including the capture and amplification of polynucleotides.

As discussed above, solid supports were widely used to isolate nucleic acids to study their structure and function or to detect the presence of a particular nucleic acid in a sample. Because the amount of a target polynucleotide in a sample is often very small, the desirability of increasing the proportional amount of isolated polynucleotide before further analysis or detection would have been obvious to one skilled in the art. It was common practice in the art to capture small amounts of mRNA on solid supports, convert the mRNA to cDNA using reverse

transcriptase, an RNA-dependent DNA polymerase, and then further amplify the cDNA by cloning prior to detecting and/or analyzing the DNA. Once methods of *in vitro* amplification became widely available, it was obvious to combine these techniques with target capture on solid supports. Moreover, because target capture was known to be less than 100% efficient, it would have been obvious to one skilled in the art to combine it with amplification procedures to increase the amount of captured target (i.e., to compensate for any losses during the capture step) before detection.

Brown et al., in "Methods of Gene Isolation" *(Ann. Rev. Biochem.,* 43:667-693 (1974)), clearly recognized the desirability of combining target capture on solid supports with amplification. The authors reviewed a number of methods for isolating nucleic acids of interest, and devoted one section to the use of polynucleotides fixed to insoluble matrices to isolate DNA. At pages 673-674, Brown et al. describe using RNA or DNA fixed to an insoluble support to effect sequence-specific isolation of target DNA containing a sequence complementary to the probe. In their "Concluding Remarks" (p. 687) Brown et al. state that purification of DNA (*e.g.* using polynucleotides fixed to insoluble matrices) could be coupled with a method by which, "...a small amount of a given gene can be increased enormously in amount" such as by an "amplification step ... carried out *in vitro* by an efficient DNA polymerase, which would replicate faithfully each molecule of DNA many times."

Arsenyan et al., *Gene* 11:97-108 (1980) also recognized the desirability of combining target capture with amplification because they described a method to produce "amplified homogeneous DNA sequences" for the purpose of studying gene arrangement. Their process captured complementary single strands of the 5S RNA gene by hybridization to probes affixed to cellulose supports followed elution of the captured strands, annealing of the strands to produce

double stranded DNA which was inserted into a cloning vector, transformation of host cells with the vector and amplification of the DNA by growing the transformed host cells. At the time of filing of the '338 patent, it would have been obvious to one of ordinary skill in the art that other known amplification methods could have been substituted for the cloning step of *Arsenyan et al.* The obviousness of such substitution is suggested by the reissue applicants' own definition of "amplify" in the Background of the Invention section of the '338 patent (col. 2, ll. 9-19). In my opinion, substituting other methods of amplification, as they became available, for amplification by cloning would have been obvious and routine to one skilled in the art.

Gaubatz et al., *Biochim. Biophys. Acta* 825:175-187 (1985) described the isolation of poly(A)$^+$ mRNA by using oligo(dT)-cellulose chromatography followed by conversion of the purified mRNA into cDNA and amplification of the cDNA using a polymerase-mediated strand displacement procedure.

Powell et al., *Cell* 50:831-840 (1987) described isolating poly(A)$^+$ RNA by one or two cycles of oligo(dT)-cellulose chromatography to capture Apo-B mRNAs followed by synthesis of cDNA and PCR amplification of the cDNA and detection of specific Apo-B48 sequences.

11. Summary of my opinion based on the state of the art at the time the '338 application was filed.

It is my opinion that the technology described in the '338 patent would have been obvious to one of ordinary skill in the art. Specifically, before the filing date of the '338 patent, it was common practice to isolate a target polynucleotide from a sample by contacting the sample with a solid support which bound to the target polynucleotide either directly or indirectly. The support with its attached polynucleotide and bound target polynucleotide was separated from

the sample, effectively separating the target from other sample components that could interfere with further manipulation of the target polynucleotide. It was also well known in the art that amplifying the target polynucleotide directly or by producing an amplified signal based upon the presence of the target polynucleotide could increase detection sensitivity. It is my opinion that it would have been obvious to one of ordinary skill in the art in December 1987 to perform the combination of those manipulations for the purpose of detecting the presence of a target polynucleotide in a sample. Based upon my understanding of the skill of the art at the filing date of the '338 patent, I conclude that one of ordinary skill would have been motivated to combine both target polynucleotide capture and amplification to ensure success in a detection assay.

12. Analysis of Dr. Persing's Declaration

I have been informed that allowance of the '338 patent followed shortly after the filing of Dr. Persing's declaration. I respectfully submit that Dr. Persing's declaration does not fully characterize the state of the art at the filing date of the '338 patent application. Further, the implicit description of the individual of ordinary skill in the art is inaccurate because it is too low. As discussed above, the skilled practioner at that time would have been aware of the different methods for target capture and amplification and would have been motivated to combine these techniques.

In my opinion, in addition to incorrectly characterizing the state of the art and of the skill level of practioners, Dr. Persing also incorrectly characterized what was known about PCR at the filing date of the '338 application. In paragraph 12 of the declaration, Dr. Persing asserts that, at the filing date, "...[T]hose who were adding amplification to their hybridization assays had a strong incentive to avoid the addition of target isolation steps to their hybridization assays". He bases this statement on his belief that users of PCR believed that PCR was so highly specific that

additional steps for isolating the target were not needed. Dr Persing stated that "It was not until much later that it became apparent that non-specific amplification was occurring despite the careful selection of primers." Dr. Persing was incorrect in stating that the problem of non-specific amplification in PCR had not been recognized by December, 1987, because the PCR inventor himself discussed the problem of "background" amplification[1]. Even if Dr. Persing's statement is accepted as correct, it does not, in my opinion, justify a conclusion that those skilled in the art had no incentive to combine target capture and amplification as described and claimed in the '338 patent.

I have carefully reviewed the specification and claims of the '338 patent and found that PCR amplification is never mentioned or described. Likewise, there is no mention of problems with PCR and no teaching, nor showing, that a combination of target capture and PCR amplification provides any improvement over the PCR process alone. Unlike PCR, all of the amplification methods disclosed in the '338 patent are non-specific amplification methods which would be expected to amplify indiscriminately any polynucleotides present in a sample. Contrary to Dr. Persing's conclusion, one skilled in the art would have had a strong incentive to isolate the target sequence before amplification, as described in the '338 patent, to avoid non-specific amplification of non-target sequences. Thus, for example, one would be motivated to

[1] In U.S. Patent No. 4,683,202, which is directed to the PCR process, inventor Mullis recognized that non-specific amplification of non-target sequences (background amplification) could be a problem, particularly when one is attempting to amplify a single-copy gene. In Example 10, he describes overcoming this problem through the use of nested primers. He also states (col. 5, ll. 34-37) that the starting nucleic acid may be in a purified form. His recognition of the problem directly contradicts Dr. Persing's statement in paragraph 12 of the declaration. Mullis described one approach to overcoming the problem. Having recognized the problem, it is my opinion that other approaches would have been obvious to the average skilled worker, including purification of the target prior to amplification using art-known techniques such as target capture on a solid support.

combine the art-known methods of isolating target polynucleotide on solid supports with the known amplification methods disclosed in the '338 patent.

In paragraph 13 of Dr. Persing's declaration, he states that practitioners of hybridization assays were reluctant to use hybridization techniques to purify their intended targets prior to amplification because the lack of complete binding efficiency of probe to target would result in a reduction of available target, which might be at a low initial concentration in the sample. In my opinion, inefficient binding of capture probe to target does not justify a conclusion that those skilled in the art would be led away from combining target capture and amplification. Although capture methods were not expected to be 100% efficient and the total number of target polynucleotides in an enriched sample would be somewhat reduced (i.e., some would not be captured), the published levels of amplification far exceeded the amount lost during target capture. For example, one might expect to capture only 50 % of target molecules, but amplification typically resulted in a 100-fold increase in the amplified sequences. Therefore, target capture of 50% and 100-fold amplification would result in a 50-fold overall increase in the number of target polynucleotides compared to those in the original sample.

Because of the anticipated loss of target during the capture step, one skilled in the art would have been *motivated* to amplify the captured target prior to detection, thereby increasing the sensitivity of the assay. Brown et al., *supra*, Gaubatz et al., *supra*, and Powell et al., *supra*, all clearly recognized that target capture combined with amplification would be advantageous for providing adequate amounts of the target polynucleotide for analysis.

In paragraph 14 of Dr. Persing's declaration, he states that the claimed invention provided an additional advantage that was unexpected before the filing date, *i.e.* the elimination

of amplification inhibitors present in the sample. In my opinion, one of ordinary skill in the art would have been fully aware before the filing date that isolation of target polynucleotide on a solid support would have advantages, including changing buffer (medium) components and removing substances that would inhibit amplification of the target polynucleotide. The presence of inhibitors of restriction endonucleases or of DNA or RNA polymerases (used in amplification reactions) in biological, environmental, or clinical samples was well known in the art before 1987. Consequently, before then it was standard practice in molecular biology to purify nucleic acids before restriction endonuclease cleavage or polymerase-mediated amplification. It would have been obvious to one skilled in the art that nucleic acid purification, *e.g.* using the known technique of target capture on a solid support, could be desirable before any amplification step which employs a polymerase because it would remove potential inhibitors of the amplification reaction. Exemplary references that discuss enzymatic inhibitors and the need for nucleic acid purification include DNA Synthesis, Kornberg, A., (Freeman & Co., San Francisco 1974) p. 65; Maniatis et al., *supra,* Chapters 6 and 7, pp. 182-196 and 213.

13. Summary of my opinion regarding Dr. Persing's Statements and Conclusions

Based on my knowledge of the state of the art at the time the '338 application was filed, the level of skill of an ordinary practitioner of molecular biology at that time, and the references discussed herein, I believe that Dr. Persing incorrectly characterized the state of the art relevant to the claimed invention of the '338 patent and further incorrectly characterized known aspects of PCR amplification.

14. The '338 patent specification describes methods that are different than those claimed in new claims 41-59.

I have been informed that the patent statutes require that the invention claimed in a patent be described in the patent specification in a manner that reasonably conveys to one of ordinary skill in the art that the inventor was in possession of the invention, as claimed, at the time of filing of the application. I have carefully read the specification and claims of the '338 patent, and it is my opinion that one skilled in the art would conclude from the specification that the inventors were not in possession of the invention of claims 41-59. At most, the specification conveys that the inventors were in possession of a method in which target capture was combined with an *in vitro non-specific* amplification step.

Claims 41-59 are directed to methods in which the amplification step is conducted *in vitro*. None of these claims is further limited to amplification that is non-specific. In the Preliminary Amendment filed with the reissue application, applicants referred to the Examples as support for these new claims. However, the *only* methods of amplification described in the Examples (as well as in the Summary of the Invention and Detailed Description, col.15, l. 56-col. 16, l. 29) are non-specific amplification methods, *i.e.* methods that indiscriminately amplify any nucleic acids present in a sample. Based on the Summary of the Invention, Detailed Description and the Examples, I believe that one skilled in the art would have thought that the only invention that the applicants possessed was one that combined target capture with non-specific amplification methods.

This is further supported by the description at column 30, lines 30-40 of the '338 patent in which the applicants indicate that a benefit of their invention is that it allows the use of non-specific amplification, i.e., the same enzymes and primers can be used for all target sequences. Those skilled in the art at the filing date of the '338 patent would have appreciated that this would have made the assay simpler and less expensive because individual primers would not have to be designed, synthesized and tested for each target (as, for example, in PCR). Specifically, the applicants stated:

> Amplification of the target nucleic acid sequences, because it follows purification of the target sequences, can employ non-specific enzymes or primers... Thus no specifically tailored primers are needed for each test, and the same standard reagents can be used, regardless of targets.

The examples in the specification that describe methods combining target capture with amplification (i.e., Examples 4 to 7) describe several different methods of amplification. The common feature of all of those methods is the non-specific nature of the amplification, a point that is repeatedly emphasized in the specification.

It is also important to note that, although PCR, a primer/target-specific amplification was well known by December 1987, the applicants did not include it in their examples or reference PCR elsewhere in the specification. This is consistent with the conclusion that the inventors only possessed methods that combined target capture with non-specific amplification. Further, no other sequence-specific amplification method is included in the description of the invention and the specification teaches the benefit of using amplification methods that do not require specific primers.

In summary, it is my opinion that one of ordinary skill in the art would conclude from the specification of the '338 patent that the only method the inventors possessed that combined *in vitro* amplification with target capture was a method that used non-specific amplification. Accordingly, claims 41-59 must include a limitation to non-specific amplification or they will be inconsistent with the applicant's disclosed invention, as one skilled in the art would understand it from the specification.

15. Conclusion. It is my opinion that the invention claimed in the '338 patent and its reissue application was known or obvious to one of ordinary skill in the art of molecular biology at the time of the '338 patent application, and that this patent should not be reissued. Further, I believe that the statements and conclusions made in Dr. Persing's declaration of 1997 should not be relied upon as an accurate characterization of the state of the art at the filing date of the '338 patent application. Finally, it is my opinion the 09/533,906 reissue application fails to describe the invention of claims 41-59 because those claims are not limited to the use of non-specific amplification, as described in the specification.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code.

July 31, 2000
Date

Michael M. Harpold